Brazilian Journal of Poultry Science
Revista Brasileira de Ciência Avícola

ISSN 1516-635X 2021 / v.23 / n.4 / 001-008

http://dx.doi.org/10.1590/1806-9061-2020-1422

Original Article

Microsatellite Polymorphism and Prokaryotic Expression of Mef2d in Xingyi Duck

■Author(s)

Xincai S[I] https://orcid.org/0000-0001-6178-5065
Hui L[I] https://orcid.org/0000-0002-5298-3657
Zhonghai Z[II] https://orcid.org/0000-0002-1874-7626
Xiaoyan B[I] https://orcid.org/0000-0001-9802-5166
Lin C[I] https://orcid.org/0000-0002-3739-9671
Huating Y[I] https://orcid.org/0000-0002-5041-2367
Xingcai L[I] https://orcid.org/0000-0002-4324-9258

[I] Guizhou University College of Animal Science/ Key Laboratory of Animal Genetics, Breeding and Reproduction in the Plateau Mountainous Region (Guizhou University), Ministry of Education, Guiyang 550025, China; Key Laboratory of Animal Genetics, Breeding and Reproduction in Guizhou Province, Guiyang 550025, China.
[II] Zunyi Animal Husbandry Station, Zunyi 563000, China.

■Mail Address

Corresponding author e-mail address
Li Hui
Guizhou University College of Animal Science/ Key Laboratory of Animal Genetics, Breeding and Reproduction in Guizhou Province, Guiyang 550025, China.
Phone:
Email: ellenlihui@sina.cn

■Keywords

MEF2D gene, microsatellite polymorphism, prokaryotic expression, slaughter traits, Xingyi duck.

Submitted: 31/May/2021
Approved: 27August/2021

ABSTRACT

Myocyte enhancer factor 2D (MEF2D) are members of the myocyte enhancer factor 2 (MEF2), a supergene family and are thought to be related to the development and regeneration of skeletal muscle. We selected a microsatellite locus located in the *MEF2D* gene to study the slaughter characteristics of Xingyi duck and discuss whether the locus could be used as a molecular genetic marker associated with the slaughter characteristics. To further study the function of this gene, we cloned the coding region of the *MEF2D* gene and expressed it in the prokaryotic expression system. We amplified exon 9 of *MEF2D* gene by PCR and analyzed after sequencing. The entire CDS region was amplified by RT-PCR. The prokaryotic expression vector was constructed by double enzyme digestion. Results showed that there was a significant correlation between the microsatellite polymorphism of exon 9 of the *MEF2D* gene and the eviscerated weight rate of Xingyi duck ($p<0.05$). The eviscerated weight rate of the aa (40/40) genotype was significantly higher than that of the ab (40/49) genotype. The CDS region of the *MEF2D* gene was cloned with a length of 1557 bp. The prokaryotic expression vector pET32a(+)-MEF2D was constructed. The results provide a foundation for future studies examining the function of the *MEF2D*.

INTRODUCTION

Myocyte enhancer factor 2 belongs to the MADS-box transcription factor family and has four members in vertebrates: *MEF2A*, *MEF2B, MEF2C* and *MEF2D* genes (Black & Olson, 1998). The lack of any member of *MEF2* family could results in the inhibition of transcription of muscle development related genes (Molkentin & Olson, 1996). The *MEF2* family not only plays an important physiological role in the occurrence and development of skeletal muscle (Zhao *et al*., 2011), but it also plays an important regulatory role in the regeneration of skeletal muscle (Liu *et al*., 2014). The important role of the *MEF2* family has attracted researchers' attention. Studies have shown that the *MEF2D* gene polymorphism is highly correlated with the growth and slaughter performance of animals. Indeed, several QTL loci related to carcass traits and growth rate have been located in exons 4–5 and intron 4 of the porcine *MEF2D* gene (Wagenknecht *et al*., 2003). AciI site in intron 4 of the porcine *MEF2D* gene may be related to carcass and meat quality traits (Chen, 2005). The *MEF2D* gene is significantly correlated with the density and diameter of three muscle fibers in goats (Cheng, 2012). The *MEF2D* gene had SNPs that were significantly related to the lean meat rate of cattle (Juszczuk-Kubiak *et al*., 2013). A microsatellite polymorphism has been identified in exon 9 of the *MEF2D* gene with CAG as a repeat unit, which had a significant correlation with the breast muscle weight

of Jianchang duck (Hu, 2014). Another study showed that this microsatellite locus was significantly correlated with the carcass weight, eviscerated weight, semi eviscerated weight, breast muscle weight and breast muscle rate of ducks (Wang *et al*., 2016). However, the effect of this microsatellite on the *MEF2D* gene CDS region and protein expression remains unclear.

Generally, the *MEF2D* gene plays an important role in the regulation of the occurrence, development and regeneration of animal muscle and muscle growth and thus can affect the slaughter traits of livestock and poultry. We detected and analyzed the polymorphism of microsatellite loci of the *MEF2D* gene and its correlation with slaughter traits in Xingyi duck. Furthermore, to understand the effect of this microsatellite site on the expression of the *MEF2D* gene, we cloned the CDS region sequence of the *MEF2D* gene and constructed the prokaryotic expression vector pET32a (+) - MEF2D. This study provides a basis for further study on the function of related genes and whether this locus can be used as a molecular genetic marker associated with slaughter traits in ducks.

MATERIALS AND METHODS

Animals and test material

All animal work was approved by the Guizhou University Subcommittee of Experimental Animal Ethics. Xingyi ducks were raised in a free-range environment. Commercial feed was provided in the morning and evening; all ducks had free access to food and water. Ducks were euthanized by electrical stunning followed by exsanguination. Leg muscle samples of 90-day-old Xingyi ducks (52 ducks) were selected for genomic DNA extraction. Breast, leg and myocardial tissue samples of 55-day-old Xingyi ducks were randomly selected for total RNA extraction, followed by cloning into pMD18-T and then constructed into the prokaryotic expression vector pET32a (+).

Genomic DNA and total RNA extraction

Genomic DNA was extracted by a DNA extraction kit (Tiangen Biochemical Technology Co., Ltd., China); OD value and concentration were detected by NanoDrop 2000 (Thermo). Total RNA was extracted by the Trizol (Thermo Fisher Science, USA) method; OD value and concentration were detected by NanoDrop 2000, and the first chain of DNA was synthesized by a RevertAid First Strand DNA Synthesis (K1622) kit (Thermo Scientific).

Identification of CAG repeats of the *MEF2D* gene in Xingyi duck

Primers were designed and synthesized for exons 9 and 10 (including the microsatellite region) according to the *MEF2D* gene sequence of Peking duck in GenBank (login number: NW_004677191.1) (Forward primer: 5′-CTCACGTTGACGTTGTGCTG-3′, Reverse primer: 5′-ACCGCTCTCTGTAGTCACCT-3′, 575 bp, Tm 65 °C). Genomic DNA was used as a template for PCR amplification and sequencing. Next, 1% agarose gel electrophoresis was used to detect the amplified products. The qualified samples were sent to the Sangon Biotech (Shanghai) Co., Ltd. for direct sequencing.

DNA Star software was used to compare the sequencing results to determine the repetition number of microsatellites (CAG). Popgene software was used to calculate the genetic information of genetic polymorphism in the population. SPSS 19.0 was used to analyze the correlation between genetic polymorphism and slaughter traits, and the results were expressed in the form of mean ± standard deviation.

Construction and identification of the prokaryotic expression vector of the *MEF2D* gene

According to the mRNA sequence of duck *MEF2D* (KT876438.1) published by GenBank, *Bam*H I and *Hind* III recognition sites were added to the 5′ of the forward and reverse primers of the CDS region to synthesize primers (Table 1).

The CDS region of the *MEF2D* gene was amplified by PCR, and cDNA was used as the template. MEF2D-CDS (1) Primer was used first, followed by amplification with MEF2D-CDS (2). Primers were used to add the restriction endonuclease recognition site and the protecting base. The PCR products were detected with

Table 1 – Primer sequence of CDS region of *MEF2D* gene.

Primer name	Primer sequence (5′→3′)	Products length/bp	Tm/°C
MEF2D-CDS (1)	F: TTTCCCGAGAAGATGGGGAG R: CCGTTATGTGACCCAGGTATCC	1 567	59
MEF2D-CDS (2)	F: CGGGATCCATGGGGAGAAAAAAGATCCAGATCC R: CCCAAGCTTTTATGTGACCCAGGTATCC	1 557	59

Note: The shaded port is the added protecting base, and the underlined part is the recognition site of restriction endonuclease.

1.0% agarose gel, and the products were recovered and purified. The purified PCR products were ligated into the pMD18-T vector (Takara Biomedical Technology (Beijing) Co., Ltd.) and then transformed into *DH5α* competent cells. Positive clones were screened by PCR in bacterial solution, and the positive clones were sent to Sangon Biotech (Shanghai) Co., Ltd. for bidirectional sequencing.

The plasmids were extracted from the identified bacterial solution and pET32a (+) empty vector and were digested with *BamH* I and *Hind* III, respectively. The total reaction system was 40 μL: the recombinant plasmid/pET32a (+) empty vector plasmid was 20 μL, *Bam*H I was 4 μL, 10x Green Buffer was 4 μL, and ddH_2O was 8 μL. The mixture was centrifuged briefly after mixing, which was done in a water bath at 30 °C for 2 h and then mixed with Hind III at 37 °C for 2 h. The target fragments of the purified recombinant plasmid and pET32a(+) empty vector were recovered, which were connected at 16 °C overnight. The products were then transformed into *DH5α* competent cells. The positive clones were screened by PCR of the bacterial solution and double enzyme digestion. Next, they were sequenced at the Sangon Biotech (Shanghai) Co., Ltd. The plasmids of pET32a (+) empty body and pET32a (+) - MEF2D were extracted and transferred into BL21 (DE3) competent cells by heat shock and were cryopreserved in glycerol at -80 °C.

Extraction of total protein and SDS-PAGE analysis

First, we resuscitated the cryopreserved strains. The next day, the cells were inoculated into 800 mL of LB liquid medium (containing Amp) at the ratio of 1:100. The cells were then cultured in a shaker at 37 °C and 200 rpm until OD_{600} was 0.6–0.8 (turbidity, usually 2–3 h). IPTG with a final concentration of 0.1 mM was added, followed by continuous culture for 4 h. The supernatant was discarded after centrifugation for 10 minutes by 6000 rpm at 4 °C . The precipitate was then resuspended in the PBS wash buffer and centrifuged for 10 minutes by 5000×g at 4 °C . We discarded the supernatant and washed the precipitate 2–3 times until the supernatant was transparent. We removed the supernatant and freeze-thawed it twice at -80 °C , followed by resuspension with 30 mL of PBS on ice. The bacteria were broken twice by a high-pressure cell breaker. The supernatant and precipitation were separated by centrifugation for 20 minutes by 8000 ×g at 4 °C . The precipitation was resuspended with a small amount of PBS and stored at -80 °C. The empty carrier bacterial solution was extracted by a bacterial protein extraction kit (Kangwei Century).

Protein samples and protein sample buffer were mixed proportionally, and the mixed liquid was boiled for 15 minutes. Fifteen μL of samples was used for SDS-PAGE (5% concentration gel, 11% separation gel). The voltage was 80 V for 30 minutes. The voltage was then raised to 120 V until bromophenol blue reached the bottom of the separation gel, and the power was turned off (usually 60–90 minutes). Protein expression was analyzed after staining and decolorization with Coomassie brilliant blue.

Western blot detection of recombinant protein

After SDS-PAGE, the protein bands were transferred to PVDF membrane (30 s activated in methanol) by Bio-Rad Mini transmembrane system (wet rotation) at 80 V. The duration was 80 minutes. The western blot assay was performed per the instructions in the WB kit. Finally, 0.05 mL of ECL working fluid under dark conditions was added per 1 cm^2 of membrane; we made direct observations in the machine after 2 minutes, followed by photography (60–300 s, 10 pictures).

RESULTS

Identification of CAG repeats of *MEF2D* gene in Xingyi duck and its correlation with slaughter traits

Amplification of the MEF2D gene by PCR

The amplified products of the microsatellite region PCR of the *MEF2D* gene in Xingyi duck were detected by 1.0% agarose gel electrophoresis (Fig. 1a). The bands were bright and single, which were consistent with the expected length of the target fragment.

Analysis of sequencing results

PCR products were compared with the *MEF2D* gene sequence published in GenBank (NW_004677191.1). Six genotypes, including aa (40/40), ab (40/49), ac (28/40), ad (38/40), ae (39/40), and ff (47/47), were found in the experimental duck population. Homozygous and heterozygous sequences are shown in Fig. 1b and Fig. 1c. The different numbers of CAG repeats in sister chromatids lead to obvious nesting peaks in heterozygote individuals. The downstream sequence was conservative, which could be used to determine the number of CAG repeats. In addition,

the sequence has redundant nesting peaks because of the existence of shadow bands.

Analysis of sequencing results

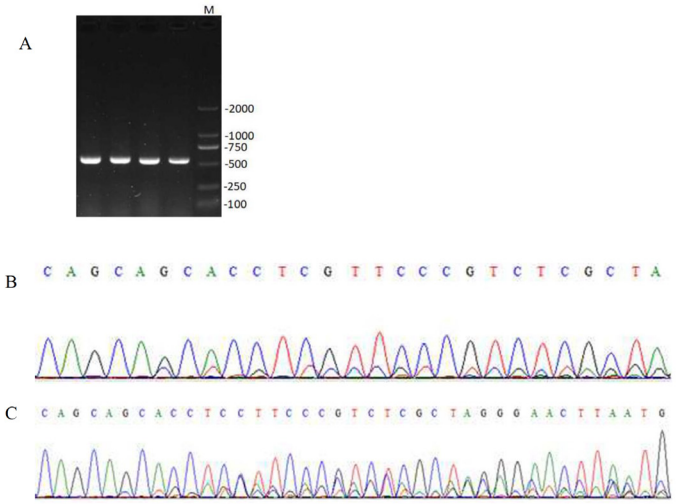

Figure 1 – Identification of CAG repeats of the MEF2D gene. a. Agarose gel electrophoresis detection of PCR amplified products from microsatellite region of MEF2D gene. b. The sequencing diagram of the homozygote. c. The sequencing diagram of the heterozygote.

Analysis of genetic polymorphism of polymorphic loci

Popgene software was used to calculate the genetic information of genetic polymorphism in the Xingyi duck population (Table 2). Six genotypes were detected in Xingyi ducks— aa, ab, ac, ad, ae, and ff—among which the frequency of aa and ab genotypes was 0.7692 and 0.1346, respectively (Table 2). The Xingyi duck population has low polymorphism (*PIC* < 0.25).

Association between *MEF2D* microsatellite polymorphism and slaughter traits

Because the numbers of ac, ad, ae, and ff genotypes detected in Xingyi ducks were too small to meet the statistical requirements, only aa and ab genotypes were used in the correlation analysis of the duck *MEF2D* gene and slaughter performance.

The averages and standard deviations of slaughter traits for each genotype of Xingyi duck are shown in Table 3. There were significant differences in the microsatellite polymorphism of exon 9 of the *MEF2D* gene and the eviscerated weight rate of Xingyi duck ($p<0.05$). The aa (40/40) genotype was significantly higher than the ab (40/49) genotype, but there was no significant difference in other slaughter traits.

Construction and identification of pET32a(+)-MEF2D prokaryotic expression vector

The amplified products of the CDS region of the *MEF2D* gene were detected by 1.0% agarose gel electrophoresis (Fig. 2b). The size of the target bands was consistent with expectations. After purification, the product was connected with the pMD18-T vector and transformed. The bacterial liquid PCR results (Fig. 2c) showed that the target stripe was neat and bright and was consistent with the expected size. Sequence alignment results (Fig. 2a) showed that the sequence of the inserted fragment was basically the same as that of Beijing duck in GenBank, except that 21 bp (the entire exon 7) was missing. The CDS region of the *MEF2D* gene contained microsatellite sequences. In this experiment, the length of the CDS region was 1557 bp, and 40 CAG repeats were obtained.

The recombinant plasmids of pMD18-T-MEF2D were digested by *Bam*HI and *Hind*III and were identified by 1.0% agarose gel electrophoresis. The results showed that there were 2 bands approximately 2692 bp and 1557 bp (Fig. 2d), which coincided with expectations. The purpose of the recovery and purification was to connect and transform with pET32a (+) vector. The bacterial solution was tested by PCR (Fig. 2e), double enzyme digestion (Fig. 2f) and sequence analysis. Sequencing results showed that the sequence of the *MEF2D* gene of Peking duck in GenBank was basically the same as that of the insertion fragment of the T vector. This finding indicated that the cloned fragment was the CDS region (1557 bp) of the *MEF2D* gene of Xingyi duck and that the prokaryotic expression vector had been successfully constructed.

Table 2 – Distribution of the frequency and heredity in different genotypes of Xingyi duck.

Type	Genotype	Genotype frequency	Allele	Allele frequency	Ho	He	Ne	PIC
40/40	aa	0.7692(40)	a	0.8750	0.7885	0.2115	1.2969	0.2204
40/49	ab	0.1346(7)	b	0.0673				
28/40	ac	0.0385(2)	c	0.0192				
38/40	ad	0.0192(1)	d	0.0096				
39/40	ae	0.0192(1)	e	0.0096				
47/47	ff	0.0192(1)	f	0.0192				

Note: The number in brackets of Genotype frequency value refers to the number of individuals.

Table 3 – Significance analysis of different genotypes of *MEF2D* gene and slaughter traits in Xingyi duck.

Genotype	Body weight (kg)	Carcass weight (kg)	Semieviscerated weight (kg)	Eviscerated weight (kg)	Leg muscle weight (kg)	Breast muscle weight (kg)	Dressing percentage (%)	Semieviscerated weight rate (%)	Eviscerated weight rate (%)	Leg muscle rate (%)	Breast muscle rate (%)
aa (40/40)	1.724±0.153	1.544±0.144	1.396±0.147	1.213±0.139	0.163±0.020	0.151±0.040	0.895±0.012	0.809±0.022	0.702±0.027*	0.135±0.016	0.123±0.023
ab (40/49)	1.699±0.181	1.521±0.192	1.353±0.161	1.149±0.144	0.155±0.015	0.132±0.048	0.894±0.020	0.797±0.047	0.676±0.031	0.137±0.024	0.113±0.028

Note: Data columns marked by * indicate significant differences ($p<0.05$) in slaughter traits between genotypes.

Construction and identification of pET32a(+)-MEF2D prokaryotic expression vector

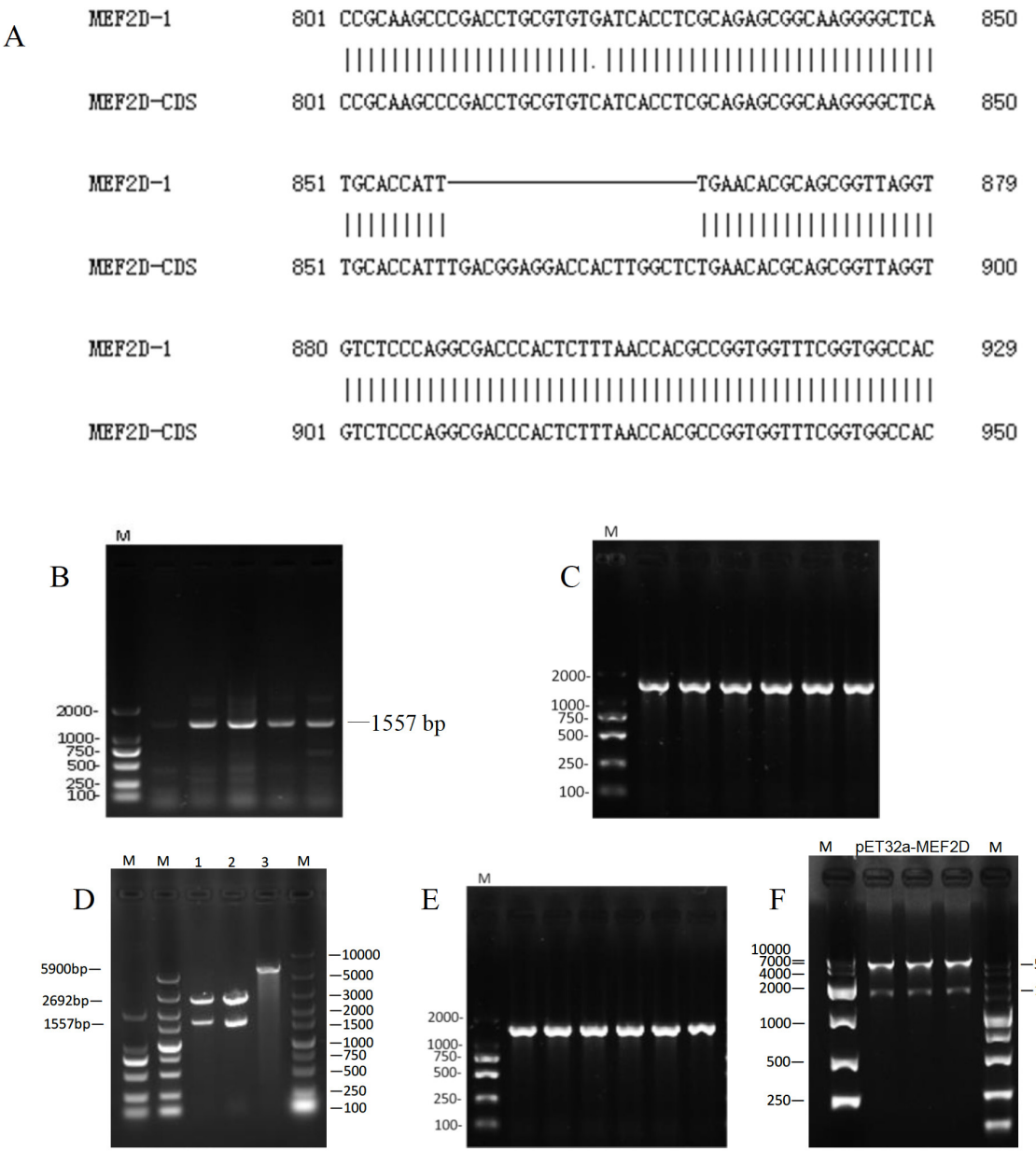

Figure 2 – Construction and identification of pET32a(+)-MEF2D prokaryotic expression vector. a. Comparison of the sequence alignment of MEF2D gene in Xingyi duck and Beijing duck. b. Agarose gel electrophoresis detection of amplified products in CDS region of MEF2D gene. c. The bacterial liquid PCR results of pMD18-T-MEF2D. d. Double enzyme digestion detection of pMD18-T-MEF2D recombinant plasmid and pET32a(+) vector plasmid. e. The bacterial liquid PCR results of pET32a(+)-MEF2D. f. Results of the double digestion of the recombinant plasmid pET32a-MEF2D.

Expression of recombinant protein

The extracted supernatants and precipitated proteins were analyzed by SDS-PAGE, and the results are shown in Fig. 3. The results showed that the protein concentration in the supernatant was higher than that in the precipitate.

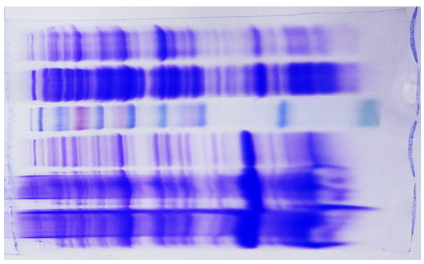

Figure 3 – The SDS-PAGE gel electrophoresis detection of supernatant and the precipitate protein. Induction conditions: 0.1 mM IPTG, 37 °C, 4 h. 1, 2: blank group supernatant; 3: blank group precipitation; M: marker (180, 130, 95, 72, 55, 43, 34, 26, 17, 10); 4: MEF2D protein supernatant; 5: MEF2D protein precipitation.

Detection of recombinant protein by Western blot

The recombinant proteins incubated with the primary and second antibodies were stained with ECL, and the results are shown in Fig. 4. The band of the target protein indicated the successful detection of the recombinant protein by the western blot. The bands of supernatant were much shallower than those of the precipitate, and MEF2D protein existed mainly in the precipitate.

Figure 4 – The western blot of the recombinant protein MEF2D (ECL). 1, 2: supernatant, 3, 4: precipitation. The dilution ratio of primary antibody was 1:8000; the dilution ratio of the second antibody was 1:4000.

DISCUSSION

Xingyi duck is a small meat-egg duck. It has high adaptability, can easily fatten and has high meat quality. It is also an excellent poultry genetic resource in Guizhou Province, China. However, the population of Xingyi duck is currently decreasing, which makes the development of Xingyi duck worrying. Poultry genetic resources are important biological genetic resources, and they provide the germplasm basis for sustainable development of poultry breeding. Animal genetic resources are becoming increasingly scarce, and the diversity of these resources is becoming homogenized; thus, the existing poultry genetic resources are critically important to the future of poultry breeding.

Because the microsatellite loci of the *MEF2D* gene are located in the coding region of the gene, we studied whether the polymorphism of the microsatellite loci affects muscle development and slaughter performance. Six genotypes were detected in the experimental duck population. The frequency of aa (40/40) and ab (40/49) genotypes was higher, and accounted for 90% of the total. The polymorphism information content of the Xingyi duck population was lower (*PIC*<0.25), indicating that polymorphism is low. This finding may be related to the long-term closure breeding of Xingyi duck. The polymorphism of the microsatellite locus was significantly correlated with the eviscerated weight rate in Xingyi ducks ($p<0.05$). The eviscerated weight rate of the aa (40/40) genotype was significantly higher than that of the ab (40/49) genotype. Whether this marker could be used as a molecular marker requires a larger sample size.

The amplified CDS region of *MEF2D* gene had the length of 1557 bp, encoding 518 amino acids. The relative molecular weight of the MEF2D protein was predicted to be 56.40 K Da. Compared with the CDS sequence (1578 bp) of Peking duck in GenBank, the amplified sequence lacked the entire exon 7 (21 bp), which reflect an alternative splicing method of the *MEF2D* gene. The C-terminal of MEF2 gene family is an active transcriptional region, and *MEF2* family is highly variable in sequence and has multiple spliceosomes in vertebrates (Wang et al., 2001; Zhu, 2004). However, no other splicing method was detected in this experiment, which may be caused by differences in breeds. Whether other splicing methods are used requires additional research.

The CDS region of the *MEF2D* gene contains microsatellite sequences, but only 40 CAG repeats of microsatellite fragments were obtained in this experiment, which may stem from the fact that nearly all individuals contained the 40 CAG repeats. The CDS region of the *MEF2D* gene was also obtained by Hu (2014). The length of his amplification fragment was 1578 bp, and its CDS region also contained 40 CAG repeats. Whether there are microsatellite polymorphisms in the CDS region needs to be studied in larger samples and in other breeds.

In this study, a prokaryotic expression vector was constructed and transferred into *E. coli*. The MEF2D protein was obtained after IPTG induction. The results of Western blot showed that MEF2D protein mainly existed in the precipitation. The specific protein band appeared at 75 K Da was different from the predicted protein size, because the pET32a (+) vector carried Trix, His and other tags. The protein can be further purified and applied to the study of duck muscle development.

CONCLUSIONS

The microsatellite polymorphism of exon 9 of the *MEF2D* gene was significantly correlated with the eviscerated weight rate in Xingyi ducks. The eviscerated weight rate of the aa (40/40) genotype was significantly higher than that of the ab (40/49) genotype. The CDS region of the *MEF2D* gene in Xingyi ducks was cloned, and its length was 1557 bp. We found that this is a new spliceosome of *MEF2D* without exon 7 (21 bp). The prokaryotic expression vector pET32a(+)-MEF2D was constructed successfully, and the target protein was successfully induced.

ACKNOWLEDGEMENTS

The work was supported by the Open Fund Project of the key laboratory of the Guizhou Provincial Education Department (KY[2018]475).

REFERENCES

Black BL, Olson EN. Transcriptional control of muscle development by myocyte enhancer factor-2(MEF2) proteins. Annual Review Cell and Developmental Biology 1998;14:167-196.

Chen L. Study on polymorphisms of porcine myostatin gene and MADs domain transcription enhancer factor 2D gene and its influence on carcass and meat quality traits [dissertation]. Ya'an (CN): Sichuan Agricultural University; 2005.

Cheng B. Study on cloning and tissue expression of *MEF2* gene family in goat. [dissertation]. Ya'an (CN): Sichuan Agricultural University; 2012.

Hu JW. *MEF2D* gene coding region clone and association analysis between its microsatellite polymorphism and slaughter performance of duck [dissertation]. Ya'an (CN): Sichuan Agricultural University; ;2014.

Juszczuk-Kubiak E, Wicinska K, Oprzadek J. Association of polymorphisms in the bovinemyocyte enhancer factor 2D(MEF2D) gene with carcass traits of Polish Holstein- Friesian cattle. Czech Journal of Animal Science 2013;58:262-269.

Liu N, Nelson BR, Bezprozvannaya S, Sheltonb JM, Richardsonc JA, Bassel-Dubya R, et al. Requirement of MEF2A, C, and D for skeletal muscle regeneration. Proceedings of the National Academy of Sciences 2014;111:4109-4114.

Molkentin JD, Olson EN. Combinatorial control of muscle development by basic helix-loop-helixand MADS-box transcription factors. Proceedings of the National Academy of Sciences of the United States of America 1996;93(18):9366-9373.

Wagenknecht D, Stratil A, Bartenschlager H, Van Poucke M, Peelman LJ, Majzlík I, et al. Linkage and radiation hybrid mapping of the porcine MEF2D gene to chromosome 4q. Animal Genetics 2003;34:232-233.

Wang DZ, Valdez MR, McAnally J, Richardson J, Olson EN. The Mef2c gene is a direct transcriptional target of myogenic bHLH and MEF2 proteins during skeletal muscle development. Development 2001;128:4623-4633.

Wang Y, Wang J, Liu H, Zhang R, Zhang T, Gan X, et al. Discovery, Characterization, and Functional Study of a Novel MEF2D CAG Repeat in Duck (Anas platyrhynchos). DNA and Cell Biology 2016;35:398-409.

Zhu B, Gulick T. Phosphorylation and alternative pre-mRNA splicing converge to regulate myocyte enhancer factor 2C activity. Molecular and cellular biology 2004;24:8264-8275.

Zhao X, Mo D, Li A, Gong W, Xiao S, Zhang Y, et al. Comparative analyses by sequencing of transcriptomes during skeletal muscle development between pig breeds differing in muscle growth rate and fatness. PLoS One 2011;6(5):e19774.